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Porang (*Amorphophallus muelleri* B.) Indirect Organogenesis

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Abstract: *Organogenesis is regeneration mechanism on the process of organ formation derived from somatic or non-meristematic tissue on in vitro technique. Organogenesis can occur directly or indirectly. This research used porang leaves as explant, and medium MS Basal with addition such as sucrose, agar, combination of PGR's 2,4 D and NAA, and aquadest. This research method used the Completely Randomize Design (CRD) with 9 different level combination of 2,4 D and NAA such as 2,0 ppm 2,4 D + 1,5 ppm NAA (A1), 2,0 ppm 2,4 D + 2,0 ppm NAA (A2), 2,0 ppm 2,4 D + 2,5 ppm NAA (A3), 2,5 ppm 2,4 D + 1,5 ppm NAA (A4), 2,5 ppm 2,4 D + 2,0 ppm NAA (A5), 2,5 ppm 2,4 D + 2,5 ppm NAA (A6), 3,0 ppm 2,4 D + 1,5 ppm NAA (A7), 3,0 ppm + 2,0 ppm NAA (A8), 3,0 ppm 2,4 D + 2,5 ppm NAA (A9) with 3 repetitions for each combination. Quantitative data such as the percentage of explants that appeared callus and roots were tested statistically by using ANOVA to determine the significance of the effect of the treatment. Treatments that had significant results were then tested using the DMRT test with a level of α=5%. Qualitative data were observed visually and analyzed descriptively. Combination concentration treatment of 2,4 D and NAA have the significant effect on callus and root formation. The level of 2 ppm 2,4 D and 2 ppm NAA is the best treatment on callus formation process in terms of 100% callus emerge and the early day appears at 24 days after planting and the best treatment on the process of root formation in terms of percentage roots emerge 100% and the early day roots appears at 46 days after planting. Indirect organogenesis is type of of root regeneration.*
Keywords: *Organogenesis; Callus; Root; 2,4 D; NAA*

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1. INTRODUCTION

Porang has the potential to become an export commodity in the industrial and agricultural sectors because it contains carbohydrates in tubers which are used as an alternative food ingredient in *modified cassava flour* (mocaf) based [1], and glucomannan which is used as an adhesive for paper, filler for drug tablets, and cosmetics, for the chemical, pharmaceutical and cosmetic industries [2]. This increases necessity and interest in porang products in both fresh and processed forms.

The strategy to increase porang production, in the conventional way, has obstacles such as planting material for stem tubers and leaf tubers or bulbil experiencing dormancy in the dry season, with a fairly long growth and development cycle of 38-43 months [3], or about 3-4 years to producing flowers, fruits and seeds [4], and optimum seeds can only be harvested after 8-9 months of the anthesis phase [5].

Plant tissue culture using porang leaf explants, is part of the asexual plant micropropagation technique, has the advantage of producing quality seeds in large quantities, because it is more effective and efficient, in terms of time period because it does not depend on time or offseason [6].

Organogenesis is the mechanism

explant regeneration in the process of organ formation derived from somatic or non-meristematic tissue in plants. Organogenesis can occur directly or organs formed without going through the callus phase and indirectly or organs formed through the callus phase.

Callus is the aggregate of amorphous or undifferentiated cells that are actively dividing, arising from injured leaf explant tissue [7]. Callus is formed on competent explant tissue that responds to signals from PGRs, and makes cells or tissues then grow, divide and develop to form callus or a dedifferentiation process [8].

Addition of synthetic auxin hormones with strong concentrations, such as 2,4 D and NAA single or combination, can stimulate callus formation, and swelling of the injured explants and forming small bumps that occur within 8-9 weeks as a sign of the process. organogenesis [9].

2. MATERIALS AND METHODS

Explants

Porang leaves explant was obtained from culture collection in Ecophysiology and Plant Tissue Culture Laboratory, Agronomy Study Program, Faculty of Agriculture University of Jember.

Medium

The culture medium used in this research was Murashige and Skoog Basal (MS Basal) with addition such as sucrose (30 $g.l^{-1}$), agar (8 $g.l^{-1}$), combination of PGRs (*Plant Growth Regulators*) 2.4 D and NAA, and aquadest.

Method

This research method used the Completely Randomize Design (CRD). In this step, 9 different level combination of 2,4 D and NAA on medium with 3 repetitions for each combination were created and labeled with A1 until A9, as listed on Table 1.

Table 1: Combination concentration of PGR.

Teatment Sample	Level of concentration (ppm)	
	2.4 D	NAA
A1	2,0	1,5
A2	2,0	2,0
A3	2,0	2,5
A4	2,5	1,5
A5	2,5	2,0
A6	2,5	2,5
A7	3,0	1,5
A8	3,0	2,0
A9	3,0	2,5

Maintenance of explants in each treatment was carried out by subculture of explants on new media with the same content periodically every 2-3 weeks.

The Percentage of Explants forming Callus

The percentage of explants forming callus is an indication of treatment in inducing/initiating explants to forming callus which is calculated from the number of explants that forming callus with the following equation.:

$$\frac{\text{Explants forming callus}}{\text{Explants planted}} x100\%$$

The Early Day of Callus Appears

The early day of callus appears is the rapidity of treatment in inducing/initiating explants forming callus which can be seen from the increase in size, thickening, swelling, and emerge of white or greenish nodules on the injured explants, that expressed in notation day after planting.

Callus Color and Texture

Callus color was seen at the time of callus emergence about 3-8 weeks after planting that observed visually and compared using *Munsell Color for Plants Tissues* as a comparison indicator of callus color. Callus texture was observed after the en-

tire callus was formed within 3-8 weeks, to determine the type of callus based on the texture which consisted of compact callus, intermediate callus (transition of friable callus), and friable callus [10].

The Percentage of Explants Forming Root

The percentage of explants forming root is an indication of treatment in inducing/initiating explants to forming root which is calculated from the number of explants that forming with the following equation:

$$\frac{\text{Explants forming roots}}{\text{Explants planted}} x100\%$$

The Early Day of Root Appears

The early day of root appears is the rapidity of treatment in inducing/initiating explants forming root which can be seen from emergence of roots on explants or callus that regenerate and forming root, that expressed in notation day after planting

Type of Organogenesis

Organogenesis is a regeneration mechanism on forming organs derived from somatic or non-meristematic tissues. Organogenesis has 2 types, direct organogenesis (organs formed directly from explant cells without going through the callus phase) and indirect organogenesis (organs formed through the callus phase)..

Data Analysis

Quantitative data such as the percentage of explants that appeared callus and roots were tested statistically by using ANOVA to determine the significance of the effect of the treatment. Treatments that had significant results were then tested using the DMRT test with a level of α=5%. Qualitative data were observed visually and analyzed descriptively.

3. RESULTS AND DISCUSSION

Effect of Combination Concentration 2,4 D and NAA on The Percentage of Explants Forming Callus

The percentage of explants that formed a callus was observed and calculated based on the number of explants that had swelling and callus appeared in the form of a lump (nodule) on the injured or sliced explants.

Table 2: ANOVA explants form callus

	Df	Sum sq	Mean sq	F-Value	F-Table 5%
Treatment	8	4,518	0,564	5,083*	2,231
Error	18	2	0,111		
Total	26	6,518			

Note: (*) = Significant

According the results of ANOVA the number of explants formed callus (Table 2), the combination concentration of the 2,4 D and NAA had a significant effect on the percentage of the number of explants forming callus.

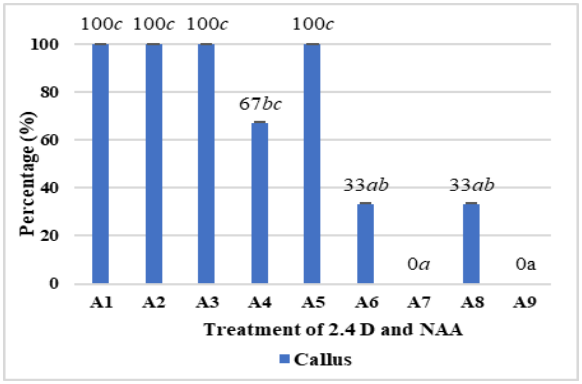

Figure 1. The effect of combination concentration 2.4 D and NAA on percentage explants forming callus

Note: The same letter are not significantly different at the 5% level of significance

Based on graphic data (Figure 1), that the difference in the percentage of explants forming callus in each treatment is thought to be due to the effect of the combination concentration of 2,4 D and NAA. Treatment A4, A6, A7, A8, and A9, it was presumed that it inhibited the response to explants and caused stagnation, because explants did not form a callus that did not reach 100%.

The treatment of PGR's concentrations can inhibit and cause stagnation, due to an imbalance between endogenous hormones (phytohormones) and the administration of exogenous hormones (growth regulators), so that the unbalanced dose can inhibit the growth of explants to form callus. [11].

Explants in stagnation conditions will remain alive but do not grow from the beginning of culture until a certain time, which can be caused by several factors, such as the condition of the media which causes a cell not stimulate to carry out the division process [12].

The higher of combination concenteration auxin PGR's added to *Amorphphallus paeoniifolius* explant the lower percentage of callus formation [13].

The Early Day of Callus Appears

The early day of callus appears begins with the appearance of callus shaped lumps (nodules) on part of explant that wounded and direct contact with the culture media.

A nodule is a callus aggregate that fused with a clustered mass that is round or globular like a lump on the edge of a leaf slice or wounded [14], have an abnormal growth and potential to develop into roots, shoots and embryoids which can then grow and develop into plantlets [15], and the initial stage of indirect organogenesis development, until the stage of shoot or root formation [16].

Figure 2. Appearance of callus in the form of nodules

The early day of callus appears was observed visually every day by calculating the average day of callus emergence in each replication in each treatment.

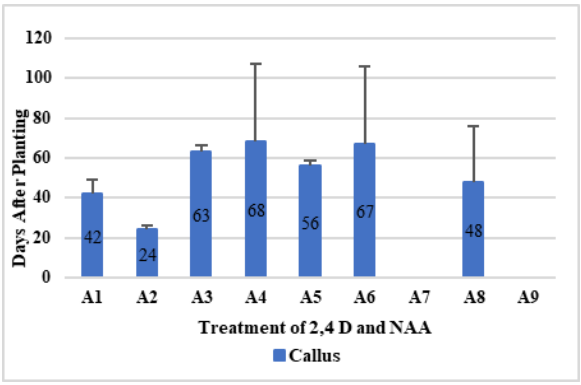

Figure 3. The effect of combination concentration 2,4 D and NAA on the average day callus emergence

According the graphic data (Figure 3), that the fastest explant response to callus formation occurred 24 days after planting on treatment A2, and the longest callus formation occurred at 68 days after planting on treatment A4. Meanwhile on the treatments A7 and A9 there were no explants forming callus due to stagnation of explants. It is also known that early explants formed callus occurred within about 24-68 days after planting or about 3-9 weeks after planting.

Differences the time period or early explants response to the treatment, were presumed from the combination concentration of 2,4 D and NAA. Conditions like this can happen because, the higher combination concenteration of 2,4 D and NAA used as

auxin-class PGR's, the longer the explants respond to treatment.

Auxin hormone 2,4 D and NAA are compounds that commonly used to cell division at levels about 0,1-50 µM because they have the advantage of being stable in heat in an autoclave, but having disadvantage due to slow failure for plant cells, so the addition of 2,4 D and NAA are sometimes added together with kinetin and BAP (cytokinin) at 0,1-10 µM levels to induce callus [17], the addition of a concentration of 2,4-D that was too high resulted in slow callus growth in small amounts, this was due to an imbalance between endogenous auxin hormones in the form of IAA (auxin), which is mostly produced on explants from apical meristem buds, young leaves and embryos in seeds [18].

Callus Color and Texture

Observation of callus color and texture, which is part of callus morphology, was carried out 3-9 weeks after planting using a light microscope for microscopic observations. The results of visual observations on callus color were then compared with the color chart (score) on the Munsell color chart for plant tissues, then the results were observed and identified to determine callus color and texture (Table 3).

Table 3: Callus Color and Texture

Treatment	Color	Score	Texture
A1	Yellow-Brown	2.5 Y 6/6	Compact
A2	Yellow	2.5 Y 8/8	Intermediet
A3	Yellow	2.5 Y 8/6	Intermediet
A4	Yellow	5 Y 8/6	Intermediet
A5	Yellow-Brown	2.5 Y 7/4	Compact
A6	Yellow	2.5 Y 8/6	Intermediet
A7	-	-	-
A8	Yellow	5 Y 8/10	Compact
A9	-	-	-

Note: (-) = Stagnation

Differences in color and texture callus indicate different phases of growth and development of cells or tissues as well as regeneration power in forming organs. Commonly, on young and actively dividing tissue the callus is white and on mature tissue it is yellowish white, meanwhile the callus texture with granules or nodules form and a clear color, has a higher ability to form organs, compared to callus that compact and brown-black color [19].

Callus with a compact texture has a dense structure and is difficult to separate, while callus with an intermediate texture is a callus that has a texture partly compact and partly friable [20]. Friable callus and white color will be embryogenic and the cell division will faster than compact callus [21], while callus compact formed because the lignification process on callus formation, so that the callus has a hard texture [22].

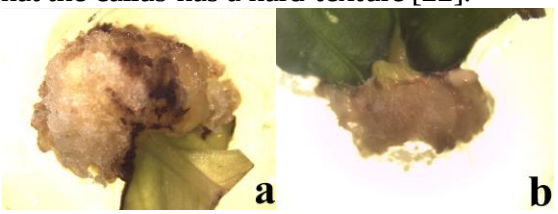

Figure 4. (a) Callus with yellow color and intermediate texture on treatment A6 (b) Callus with yellow-brown color and compact texture on treatment A5

Effect of Combination Concentration 2,4 D and NAA on The Percentage of Explants Forming Roots

The percentage of explants forming roots was observed and calculated based on the number of explants that had formed callus and developed into roots on the part of explants that wounded or sliced.

Table 4: ANOVA explants forming roots

	Df	Sum sq	Mean sq	F-Value	F-Table 5%
Treatment	8	5,629	0,703	19*	2,231
Error	18	0.667	0,037		
Total	26	6,296			

Note: (*) = Significant

According the ANOVA results, the number of explants formed roots (Table 4), the combination concentration 2,4 D and NAA had a significant effect on the percentage explants that formed roots.

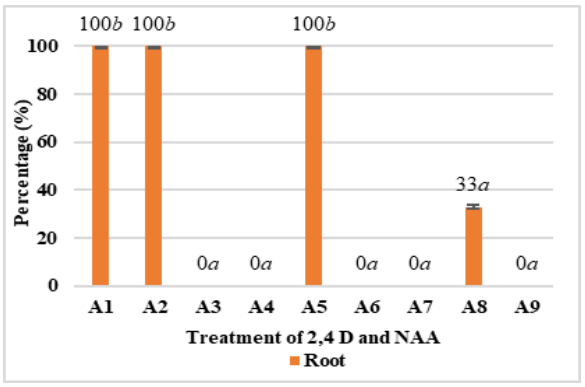

Figure 5. The effect of combination concentration 2,4 D and NAA on percentage explants forming root

Note: The same letter are not significantly different at the 5% level of significance

According the graphic data (Figure 5) that the combination concentration 2,4 D and NAA with different levels is presume have a relationship with the development of explants in forming roots. The difference can be seen in treatments A3, A4, A6, A7, A8, and A9 which inhibit the emergence of roots until stagnation. The combination of 2,4 D and NAA with higher concentrations can inhibit root formation due to an imbalance of exogenous and endogenous.

Root formation will develop at a combination of 2,4 D and NAA with low concentrations and NAA, and will be inhibited at higher concentrations, so the addition of combination concetreations 2,4 D and NAA has different roles for inducing roots depending on the species and type of explant used [23].

The combination concentration of high auxin can inhibit root induction is presumed because the combination and concentration of exogenous auxin that added cause an imbalance between endogenous auxin-cytokinin content in plant tissues (phytohormones), so that the addition of optimal concentrations is a factor that can determine whether roots are formed or not on explants [24].

The Early Day of Roots Appears

The early day of roots appears was observed visually every day by calculating the average day of roots emergence in each replication in each treatment.

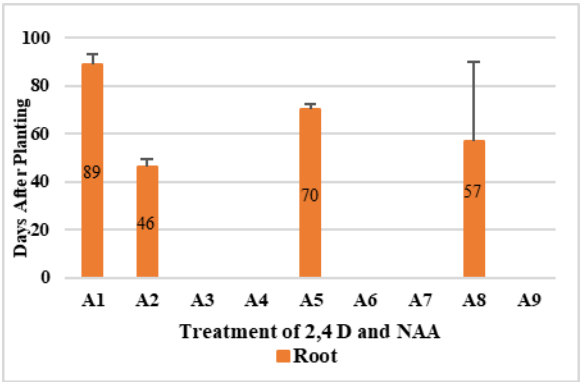

Figure 6. The effect of combination concentration 2.4 D and NAA on the day root appears

According data on the graph (Figure 3), that the fastest root formation occurred at

46 days after planting in treatment A2, and the longest occurred at 89 days after planting in treatment A1. Meanwhile treatments A3, A4, A6, A7, and A9 there were no explants in forming roots due to stagnation.

According to the graph, it is known that the early day of explants formed roots occurred about 46-89 days after planting or about 6-12 weeks after planting. The combination of auxin-cytokinin PGR's in *Amorphophallus campanulatus* B. recorded callus initiation and regeneration days around 4-12 weeks after planting. [25].

Comparison of higher concentrations of auxin hormones than cytokinins, and the use of explants with young leaves is faster in root formation [26].

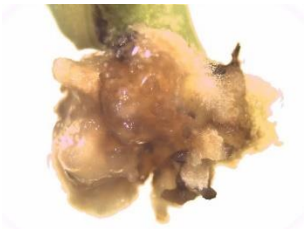

Figure 7. Roots emerge from explants that have formed callus

Type of Organogenesis

Organogenesis is the mechanism of callus development in forming roots was observed every day after planting. Observation of regeneration callus and roots by observing the explants that forming roots through the callus phase or not through the callus phase.

Table 5: Type of Organogenesis

Treatment sample	Callus	Root	Organogenesis
A1	+	+	Indirect
A2	+	+	Indirect
A3	+	-	Indirect
A4	+	-	Indirect
A5	+	+	Indirect
A6	+	-	Indirect
A7	-	-	-
A8	+	+	Indirect
A9	-	-	-

Note: (-) = Stagnation.

Based on qualitative data from observations of organogenesis mechanism variables (Table 5), that on treatments A1, A2, A3, A4, A5 and A6 organogenesis occurred indirectly because explants through the callus formation first and was an indirect organogenesis type. Meanwhile on treatments A7 and A9, callus and root formation not occurred due to stagnation. Treatments that did not form roots, but callus was formed, such as treatment A3, A4, and A6, because the treatments caused explants stagnated in the callus phase.

The different of combination concentrations of 2,4 D and NAA determined the organogenesis process on forming roots. This happens because the effect from the addition of combination concentrations treatment of 2.4 D and NAA, so that the combination of 2.4 D and NAA in low concentrations leads to an indirect process of organogenesis, while at higher concentrations it can cause stagnation on exsplan phase or callus phase.

Direct organogenesis through the stages of explants, meristemoid, primordia, and organs, while indirect organogenesis through the stages of explants, callus, meristemoid, primordia, and organs [27]. Direct and indirect organogenesis in producing adventitious shoots or roots is influenced by the combination and concentration of PGR's that used, because if the auxin ratio greater than cytokinin, it will produce roots, and if the auxin ratio is smaller than cytokinin it will produce shoots, meanwhile the formation of callus occurs when the ratio of auxin and cytokinin is 1:1 [28].

The addition of exogenous auxin and cytokinin (PGR's) can change the concentration of endogenous hormones in cells or tissues plant. The effectiveness of auxin or cy-

tokines PGR's is related to the concentration of endogenous hormones in plant tissue, it is because endogenous hormones are very abundant on young explants that have meristem tissue, that are actively growing, such as shoot tip and root [29].

Stagnation on explants or callus phase at the high of combination concentration, because the increase of auxin concentration can decrease and inhibit the formation of embryoids (non-zygotic embryos or embryo-like structures produced from somatic cells), so that with exogenous auxin (PGR's) stimulation at the right doses, the competent cells and tissues can regenerate to form organs [30].

4. CONCLUSION

Based in the results of this study concluded the following, such as:

1. The combination concentration treatment of 2,4 D and NAA have the significant effect on callus and root formation of (*Amorphophallus muelleri* B.)
2. The combination concentration of 2 ppm 2,4 D and 2 ppm NAA is the best treatment on the callus formation process in terms of 100% callus emerge and the early day appears at 24 days after planting.
3. The combination concentration of 2 ppm 2,4 D and 2 ppm NAA is the best treatment on the process of root formation in terms of percentage roots emerge 100% and the early day roots appears at 46 days after planting.
4. Regeneration of root formation occurs indirectly or type of indirect organogenesis.

REFERENCES

[1] S. S. Yuwono, K. Febrianto and N. S. Dewi, "Pembuatan Beras Tiruan Berbasis Modified Cassava Flour (MOCAF): Kajian Proporsi Mocaf: Tepung Beras Dan Penambahan Tepung Porang," *Jurnal Teknologi Pertanian,* vol. 14, no. 3, pp. 175-182, 2013.

[2] R. Sari dan S. Suhartati, "Tumbuhan Porang : Prospek Budidaya Sebagai Salah Satu Sistem Agroforestry," *Buletin Eboni,* vol. 12, no. 2, pp. 97-110, 2015.

[3] Sumarwoto, "Iles-Iles (Amorphphallus muelleri Blume); Deskripsi Dan Sifat-Sifat Lainnya," *Biodiversitas,* vol. 6, no. 3, pp. 185-190, 2005.

[4] R. Hidayat, F. D. Dewanti dan Hartojo, Tanaman Porang Karakter, Manfaat dan Budidaya, Surabaya: Graha Ilmu, 2013.

[5] M. Sari, E. Santosa, Lontoh, P. Adolf dan A. Kurniawati, "Kualitas Benih dan Pertumbuhan Bibit Tanaman Iles-Iles (Amorphophallus muelleri Blume) Asal Media Tumbuh Berbeda," *Jurnal Ilmu Pertanian Indonesia (JIPI),* vol. 24, no. 2, pp. 144-150, 2019.

[6] Ibrahim dan S. D. Meynarti, "Perbanyakan Iles-Iles (Amorphophallus Spp.) Secara Konvensional Dan Kultur In Vitro Serta Strategi Pengembangannya," *Perspektif,* vol. 18, no. 1, pp. 67-78, 2019.

[7] D. Suheriyanto, Romaidi, Resmisari dan S. Ruri, "Pengembangan Bibit Unggul Porang (Amarphopallus oncophilus) Melalui Teknik Kultur In Vitro Untuk Mendukung Ketahanan Pangan Nasional," *El-Hayah,* vol. 3, no. 1, pp. 16-23, 2012.

[8] Aziz, M. Mochammad, E. Ratnasari, Rahayu dan S. Yuni, "Induksi Kalus Umbi Iles-Iles (Amorphophallus muelleri) Dengan Kombinasi Konsentrasi 2,4-D Dan BAP Secara In Vitro," *Lentera Bio,* vol. 3, no. 2, pp. 2252-3979, 2014.

[9] M. Imelda, A. Wulansari dan Y. S. Poerba, "Regenerasi Tunas dari Kultur

Tangkai Daun Iles-iles (Amorphophallus muelleri Blume)," *Biodiversitas,* vol. 9, no. 3, pp. 173-176, 2008

[10] H. Turhan, "Callus Induction and growth in transgenic potato genotypes," *African Journal of Biotechnology,* vol. 3, no. 8, pp. 375-378, 2004.

[11] S. Sakina, S. Anwar dan F. Kusmiyati, "Pertumbuhan Planlet Anggrek Dendrobium (Dendrobium sp.) secara In Vitro pada Konsentrasi BAP dan NAA Berbeda," *Jurnal Pertanian Tropik,* vol. 6, no. 3, pp. 430-437, 2019.

[12] E. Nurcahyani, M. R. Sazilly, S. Farisi dan R. Agustrina, "Efek Inokulasi Rhizoctonia Solanii Terhadap Kandungan Karbohidrat Terlarut Total Planlet Kacang Panjang (Vigna Unguiculata (L.) Walp.) Secara In Vitro," *Analit: Analytical and Environmental Chemistry,* vol. 4, no. 1, pp. 81-90, 2019.

[13] S. Kamala dan T. Makeshkumar, "Optimization of in vitro regeneration and microcom induction in elephant foot yam (Amorphophallus paeoniifolius)," *African Journal of Biotechnology,* vol. 13, no. 49, pp. 4508-4514, 2014.

[14] Y. Rinanto, "Induksi Kalus dan Deteksi Kandungan Alkaloid Daun Jarak Jatropha curcas L.) Menggunakan Hormon 2,4-D dalam Media MS (Murashige Skoog)," *AGROVIGOR,* vol. 4, no. 1, pp. 1-6, 2011.

[15] F. R. Lina, E. Ratnasari dan W. R., "Pengaruh 6-benzylamino purine (BAP) dan 6-furfuryl amino purine (Kinetin) pada Media MS terhadap Pertumbuhan Eksplan Ujung Apikal Tanaman Jati secara In Vitro," *LenteraBio,* vol. 2, no. 1, pp. 57-61, 2013.

[16] E. Hariono, M. N. Isda dan S. Fatonah, "Pembentukan Nodul Dari Biji Manggis (Garcinia mangostana L.) Asal Bengkalis Pada Media Wpm Dengan Penambahan Bap Dan Madu," *AL-KAUNIYAH: Journal of Biology,* vol. 11, no. 1, pp. 16-2, 2018.

[17] L. R. Wetter dan F. Constabel, Plants tissue culture methods. Second Edition., Canada: Prairie Regional Laboratory, 1991.

[18] I. B. Tonga, E. B. M. Siregar dan N. Anna, "Respon Eksplan Biji Gaharu (Aquilaria malaccensis Lamk.) Terhadap Pemberian 2,4-D Secara in Vitro," *Peronema Forestry Science Journal,* vol. 2, no. 1, pp. 16-21, 2013.

[19] R. Purnamaningsih, "Induksi Kalus dan Optimasi Regenerasi Empat Varietas Padi melalui Kultur In Vitro," *Jurnal AgroBiogen,* vol. 2, no. 2, pp. 74-80, 2006.

[20] Y. Rasud dan Bustaman., "Induksi Kalus secara In Vitro dari Daun Cengkeh (Syizigium aromaticum L.) dalam Media dengan Berbagai Konsentrasi Auksin," *Jurnal Ilmu Pertanian Indonesia (JIPI),* vol. 25, no. 1, pp. 67-72, 2020.

[21] D. Hapsoro, D. Setiawan, R. Hamiranti dan Yusnita., "Pengaruh 2-iP, BA, 2,4-D, dan TDZ Pada Embriogenesis Somatik In Vitro Kopi Robusta Unggul Lampung," *J. Agrotek Tropika,* vol. 7, no. 3, pp. 527-537, 2019.

[22] I. Mahadi, W. Syafi'i dan Y. Sari, "Induksi Kalus Jeruk Kasturi (Citrus microcarpa) Menggunakan Hormon 2,4-D dan BAP dengan Metode in vitro," *Jurnal Ilmu Pertanian Indonesia (JIPI),* vol. 21, no. 2, pp. 84-89, 2016.

[23] J. J. Jeyakumar dan L. Vivekanandan, "Optimization of Conditions for Callus Induction and Indirect Organogenesis of Cucumis anguria L.," *Asian Journal of Plant Science and Research,* vol. 5,

no. 11, pp. 53-61, 2015.

[24] T. Arlianti, S. F. Syahid, N. N. Kristina dan O. Rostiana, "Pengaruh Auksin IAA, IBA, Dan NAA Terhadap Induksi Perakaran Tanaman Stevia (Stevia rebaudiana) Secara In Vitro," *Bul. Littro,* vol. 24, no. 2, pp. 57-62, 2013.

[25] K. K. Paul, M. A. Bari, M. S. Islam dan S. C. Debnath, "In vitro Shoot Regeneration in Elephant Foot Yam, Amorphophallus campanulatus Blume.," *Plant Tissue Cult. & Biotech,* vol. 23, no. 1, pp. 121-126, 2013.

[26] F. A. Prayana, Djenal dan R. Wardana, "Mikropropagasi Tangkai Daun Iles-Iles (Amorphophallus muelleri Blume) Secara In Vitro Dengan Penambahan ZPT BAP dan NAA," *Agriprima: Journal of Applied Agricultural Sciences,* vol. 1, no. 2, pp. 95-104, 2017.

[27] D. Hapsoro dan Yusnita., Kultur Jaringan (Teori dan Praktik), Yogyakarta: ANDI, 2018..

[28] R. Mastuti, Dasar-Dasar Kultur Jaringan Tumbuhan, Malang: UB Press, 2017

[29] P. N. Indah dan D. Ermavitalini, "Induksi Kalus Daun Nyamplung (Calophyllum inophyllum Linn.) pada Beberapa Kombinasi Konsentrasi 6-Benzylaminopurine (BAP) dan 2,4-Dichlorophenoxyacetic Acid (2,4-D)," *Jurnal Sains Dan Seni Pomits,* vol. 2, no. 1, pp. 2337-3520, 2013.

[30] R. Arimarsetiowati, "Pengaruh Auksin 2,4-D dan Sitokinin 2-ip Terhadap Pembentukan Embriogenesis Somatik Langsung Pada Eksplan Daun Coffea arabica L.," *Pelita Perkebunan,* vol. 27, no. 2, pp. 68-77, 2011.